Citrus Tristeza Virus in Cyprus

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ABSTRACT. A survey for citrus tristeza virus (CTV) was conducted in Cyprus from 1987 to 1991 using Mexican lime indicator seedlings. Samples were obtained from a total of 725 trees of 28 citrus species and varieties grown in 156 groves in the main citrus-producing areas of the island. Infection was detected in four groves, two of clementine, one of lemon, and one of grapefruit. When all trees in these groves were tested by ELISA, the incidence of CTV ranged from 16 to 62%. Infected grapefruit trees exhibited evident decline symptoms, whereas infected clementines showed mild symptoms and lemons were symptomless. No evidence of seedling yellows was found. There were indications that CTV is naturally spreading and an isolate from clementine was experimentally transmitted from infected to healthy Mexican lime seedlings by *Aphis gossypii*.

Tristeza was first detected in Cyprus in 1968 (7) when 27 trees of five citrus species were found infected. All diseased trees were destroyed. Since then no problem due to tristeza has been observed on the island. However, the use of the tristeza-sensitive sour orange as the main rootstock, and the severe CTV spread reported in Mediterranean countries like Spain (6) and Israel (1, 2), prompted the initiation of a survey for CTV. This paper reports the results of indexing work conducted from 1987 to 1991, and preliminary characterization of two local CTV isolates.

MATERIALS AND METHODS

Field surveys and lime indexing. Samples for indexing were obtained from trees in 156 groves located in the main citrus-producing areas of Cyprus. An effort was made to include trees of exotic varieties, and those suspected for CTV infection because of poor growth, stunting, scion overgrowth and honeycombing below the bud union. Four budsticks were collected from each tree and these were indexed on at least one Mexican lime seedling (8). Each lime seedling was graft-inoculated with 2-3 pieces of bark. In groves with healthy appearance five trees were randomly selected and composited for indexing on two Mexican lime seedlings. Mexican lime and the other indicator plants used in this work were grown in a partially shaded glasshouse in which the air temperature was 14-37 C. Inoculated plants were kept in the glasshouse for over 9 months and periodically observed for symptoms.

ELISA tests. All trees of any grove in which infection was detected by lime indexing were tested by ELISA using monoclonal antibodies (from Bioreba AG, Switzerland or from Immunologia y Genetica Aplicada, S.A., Spain) and following the procedure of Clark and Adams (5) with minor modifications according to the instructions provided by the suppliers. Plant extracts 1:10 (w/v) were prepared in buffer (phosphate saline pH 7.4 with 0.05% Tween 20 and 2% polyvinyl-pyrrolidone) using leaf midribs collected from the four quadrants of each tree.

Biological characterization of a CTV isolate. An isolate of CTV from a chlorotic and stunted clementine tree (89-197), which induced intense vein clearing, stunting and stem pitting on Mexican lime seedlings, was graft-inoculated in the glasshouse onto seedlings of sour orange and grapefruit CRC 343 and onto sour orange topgrafted with navel orange. Two plants of each kind were used for inoculation and two were left uninoculated as controls. All plants were tested by ELISA for CTV infection 6 months after inoculation and were observed for symptom development for over one year.

Aphid transmission. Two CTV isolates were tested for aphid-transmissibility: isolate 89-197 which was described above, and isolate 89-507 which came from a symptomless lemon tree

and caused mild vein clearing, very mild stem pitting and no stunting in Mexican lime. The only aphid species included in these tests was *Aphis gossypii* Glover, which is commonly found on citrus in Cyprus during spring and autumn. Adult apterae were collected from citrus field trees and reared on Madam Vinous seedlings. Both alatae and apterae aphids were transferred from Madam Vinous rearing plants to CTV-infected Mexican lime plants and were allowed an acquisition feeding period of not less than 48 hr. Twenty to 30 aphids were then transferred with a brush to Mexican lime seedlings, 20-30 cm high, and were left there for a 48 hr inoculation feeding. All plants with aphids were kept in growth rooms at 18-28 C. After the inoculation feeding the young Mexican lime plants were sprayed with an aphicide and transferred to the glasshouse. Three months later they were tested by ELISA for CTV. They were also examined weekly for symptoms for a period of about 9 months. The Madam Vinous plants, on which the aphids were reared, were tested monthly by ELISA for CTV and were never found infected.

RESULTS

Results from lime indexing tests were obtained for 725 trees of 28 citrus species and varieties grown in 156 groves. Typical CTV symptoms developed on Mexican lime seedlings inoculated with samples from four groves: two with clementines, one with an everbearing lemon variety and one with a Marsh Seedless grapefruit. These groves were situated in three different localities of the southeastern part of the island. The rootstock in all four groves was sour orange. Clementines and lemons were adjacent to each other in the Xylotymbou grove. When trees in each of the four infected groves were individually tested by ELISA, the incidence of CTV was found to be 16 to 62% (Table 1). Several trees of other citrus varieties interplanted among the main variety also indexed positive for CTV by ELISA. Contrarily, 658 trees of five varieties (mainly Valencia and Jaffa orange), located close to the clementine and lemon groves of Xylotymbou in which CTV infection was detected, indexed negative for CTV by ELISA. The Mosphiloti grove is in an isolated area with no other citrus in the vicinity. Several citrus plantings near the infected Vrysoules grove have not yet been tested.

To determine if any CTV spread could be occurring within affected groves, all trees in the Mosphiloti clementine planting were individually indexed by ELISA during April-May for three consecutive years (1989-1991). Concurrently several individual trees were also indexed on Mexican lime seedlings. Trees found infected in 1989 and 1990 were the same. However, in 1991, a clementine tree (90-179) which was previously free of CTV, was found infected by both ELISA and lime indexing.

Symptoms varied in infected field trees. Lemons were symptomless, whereas clementines showed mild to moderate chlorosis, mild stunting, off-season flowering, and thickening, downward rolling and veinal chlorosis of mature leaves. Marsh Seedless grapefruit exhibited evident decline symptoms including mild to severe stunting, chlorosis, dieback of branches and mild stem pitting. Several infected trees had a yellow to brown ring just below the bud union at the wood surface and mild inverse pitting. The bark had a brownish color and a cheesy texture. A few trees had collapsed and several others had died and had been removed. The ratio D/E (the proportion of ELISA-positive trees showing decline symptoms), which was proposed as an index of strain severity (4), had a high value (0.85) for the CTV-affected grapefruit grove in June 1991. The D/E ratio for the two affected clementine groves were lower.

Symptoms produced on Mexican lime varied from mild leaf vein clearing and mild stem pitting to intense vein

clearing, small-sized leaves with upward cupping, stunting and severe stem pitting. Budwood from lemon trees indexed to Mexican lime always induced mild symptoms. The severe isolate 89-197 produced no symptoms on sour orange and CRC 343 grapefruit indicator seedlings, but induced some thickening of mature leaves and about 25% growth reduction of navel orange budded on sour orange one year after inoculation. All plants inoculated with the 89-197 isolate reacted positively to CTV by ELISA independently of their symptom intensity, whereas uninoculated controls always indexed negative. *A. gossypii* transmitted isolate 89-197 to 4 out of 10 inoculated Mexican lime seedlings, whereas none of the four lime seedlings inoculated with isolate 89-507 was infected. The infected plants showed typical leaf vein clearing 6-8 weeks after inoculation and gave a strong ELISA reaction for CTV. The plants which remained symptomless were also negative by ELISA.

DISCUSSION

The detection of CTV in three different citrus varieties and in four groves during a survey which included less than 1000 citrus trees may be an indication that tristeza had spread in Cyprus before any actual disease problem became apparent.

The budwood for all Xylotymbou clementines, part of the Mosphiloti clementines and 86% of the Vrysoules grapefruit trees (Table 1) came from a nursery and or groves situated in the Famagusta district, where infected material from South Africa had been introduced in 1929 to establish a variety collection (7). The infected lemons came from material which was smuggled into the country from Australia. It is therefore possible that tristeza came mainly from South Africa, but there were more than one source of the disease.

The absence of CTV in more than 600 trees growing near the two CTV-affected Xylotymbou groves suggests that the virus is not readily transmitted under the Cyprus conditions. This suggestion is supported by results obtained from the Vrysoules grove. In this grove nearly all infected grapefruit trees came from a single source of budwood, whereas only one out of 100 trees, originated from a different budwood source, was found infected. However, the presence of CTV in a few trees of different varieties scattered among the main variety of the infected groves (Table 1), and one new infection found during the yearly indexing of the Mosphiloti clementine grove are indications that tristeza is spreading naturally in Cyprus, possibly at a low rate. The relatively efficient vector transmission of a CTV isolate from infected to healthy lime seedlings (4/10) is an additional indication for the natural spread of CTV. The ability of CTV to spread naturally under Cyprus conditions is not a surprise considering the wide natural spread of this virus in the neighbouring countries of Israel and Spain during the last few decades (3).

The fact that tristeza has not become evident to the present may be a result of mild virus isolates being predominant. Many trees found infected were symptomless or showed mild symptoms. The severe decline state exhibited by the infected grapefruit grove (D/E = 0.85) may have been partly due to water-stress from which the trees suffered during the last 3-4 yr as a result of drought and lack of proper irrigation, but it is also a result of mild stem pitting which was observed in several declining trees. There has not been any evidence of the presence of seedling yellows. The CTV isolate 89-197 which produced severe symptoms in Mexican lime did not induce any yellowing in sour orange or grapefruit. However, several tristeza isolates should be characterized biologically to obtain more accurate conclusions regarding the severity of local CTV isolates. In addition, a systematic survey of all citrus grown on the island needs to be conducted for determining the

extent of CTV spread. This will enable the establishment of adequate measures to control tristeza disease before it becomes destructive to the island's citrus industry.

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